ARO 18581.1-CS

INFRARED PROPERTIES OF BIOLOGICAL MATERIALS OF INTEREST TO THE ARMY

INTERIM TECHNICAL REPORT 4

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June 1984

Prepared for:

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Contract No. DAAG29-82-K-0126

Project 4504

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SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

1a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY N/A	3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release Distribution unlimited.
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE N/A	
4. PERFORMING ORGANIZATION REPORT NUMBER(S) Interim Technical Report 4	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION SRI International 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION U.S. Army Research Office
6c. ADDRESS (City, State and ZIP Code) 333 Ravenswood Avenue Menlo Park, CA 94025	7b. ADDRESS (City, State and ZIP Code) P.O. Box 12211 Research Triangle Park, NC 27709
8a. NAME OF FUNDING/SPONSORING ORGANIZATION U.S. Army Research Office 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER DAAG29-82-K-0126
8c. ADDRESS (City, State and ZIP Code) P.O. Box 12211 Research Triangle Park, NC 27709	10. SOURCE OF FUNDING NOS. PROGRAM ELEMENT NO. / PROJECT NO. / TASK NO. / WORK UNIT NO.
11. TITLE (Include Security Classification) Infrared Properties of Biological Materials of Interest to the Army	
12. PERSONAL AUTHOR(S) David E. Cooper	
13a. TYPE OF REPORT Interim Technical 13b. TIME COVERED FROM 1/1/84 TO 6/30/84	14. DATE OF REPORT (Yr., Mo., Day) June 1984 15. PAGE COUNT 20
16. SUPPLEMENTARY NOTATION	
17. COSATI CODES FIELD / GROUP / SUB. GR.	18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number) carbon dioxide

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

IR backscatter was measured from aerosols of potassium bromide, B. subtilis, B. subtilis spores, and M. luteus over the 9- to 11-micron region using a tunable CO_2 laser. The observed backscatter signatures were found to be in agreement with the predictions of Mie theory using the measured complex refractive indices appropriate to the material. Based on this work, we conclude that IR DISC is capable of detecting aerosols of biological organisms of concentrations of 100 particles/cm^3. The U.S. Army's detection goals are in the range of 5- to 10-particles/liter. Additional sensitivity can be achieved by extending the DISC technique to polarization sensitive methods.

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☒ DTIC USERS ☐	21. ABSTRACT SECURITY CLASSIFICATION UNCLASSIFIED
22a. NAME OF RESPONSIBLE INDIVIDUAL Dr. David E. Cooper	22b. TELEPHONE NUMBER (Include Area Code) (415) 859-3742 22c. OFFICE SYMBOL

DD FORM 1473, 83 APR EDITION OF 1 JAN 73 IS OBSOLETE.

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SECURITY CLASSIFICATION OF THIS PAGE

CONTENTS

LIST OF ILLUSTRATIONS iv

1. Work Plan 1

2. Progress 1

Aerosol Generation 2
IR Backscatter Measurements 2
KBr Aerosols 4
B. subtilis Spore Aerosols 6
B. subtilis Aerosols 8
M. luteus Aerosols 8
Conclusions 12

3. Future Plans 12

4. Scientific Personnel 16

A-1

ILLUSTRATIONS

1	Aerosol Generator and Equipment for Measurement of 9- to 11-micron Backscatter and Extinction from Biological Dispersions	3
2	Normalized IR Backscatter from KBr Aerosol	5
3	Normalized IR Backscatter from an Aerosol of *B. subtilis* Spores (0.1% Concentration)	7
4	Normalized IR Backscatter from an Aerosol of *B. subtilis* Spores (0.3% Concentration)	8
5	Normalized IR Backscatter from an Aerosol of *B. subtilis*	9
6	Scanning Electron Micrographs of *M. luteus* Filter (Samples Taken from Aerosol Chamber)	10
7	Normalized IR Backscatter from an Aerosol of *M. luteus* (0.1% Concentration)	11
8	Normalized IR Backscatter from an Aerosol of *M. luteus* (0.3% Concentration)	13
9	Effect of Size Distribution on IR Backscattering from Aerosols of *B. subtilis* Spores	13
10	Diagram of Equipment for Measurement of Circular-Intensity Differential Scattering from Biological Simulant Aerosols	15

1. Work Plan

The purpose of the research performed under this contract is to investigate the interaction of infrared (IR) radiation with biological materials. An understanding of the IR properties of biological materials will enable one to evaluate the feasibility of using active and passive techniques for remote and point detection and alarm. The objectives of this work are to:

(1) Measure the real and imaginary indices of refraction of Escherichia coli, Bacillus subtilis, Micrococcus luteus, Paracoccus dentrificans, tryptophan, egg growth medium, and casein partial hydrolysate over the wavelength range from 2 to 14 microns.

(2) Establish a data base of measured backscatter and extinction versus wavelength for the above materials in aerosol form.

(3) Compare the measured backscatter and extinction signatures with values calculated using standard spherical and nonspherical particle theories to test their accuracy in calculating IR properties of biological aerosols.

(4) Investigate the ability to discriminate biological particles from nonbiological particles by observation and suitable processing of scattering and extinction data.

2. Progress

During the six-month period ending 30 June 1984, we have concentrated our efforts on the measurement of infrared backscatter from aerosols of B. subtilis, B. subtilis spores, and M. luteus. Backscattering spectra were obtained in the 9- to 11-micron region using a grating tunable CO_2 laser. The optical system used for the measurements was calibrated using the backscattering signature from aerosols of potassium bromide (KBr). Finally, normalized backscattering data from our biological aerosol samples were compared with theoretical values calculated using a Mie scattering code and the measured complex refractive indices and size distributions for these materials.

Aerosol Generation

A schematic of the equipment used in the aerosol measurements is shown in Figure 1. The biological material to be examined was obtained as a suspension in water from the SRI Biomedical Research Laboratory, and diluted with distilled water to the appropriate concentration. Most of the samples received contained about 1 gram of biological material, and measurement time and aerosol flow constraints required that we start with approximately 1 liter of solution. Therefore, most of our aerosol measurements were made using solutions of 0.1% concentration by weight. Backscattering measurements were completed typically within 3 hours after receipt of the sample.

The aerosol was generated by the following procedure: A dilute solution of biological material was fed into a pneumatic atomizer by a syringe pump at a rate of 7.6 cm^3/min. The atomized sample was then mixed in an aerosol generator column with 50 C drying air to remove the liquid water and form a dry aerosol of biological material. Next, the aerosol was fed into the scattering chamber and finally exhausted to the atmosphere through a HEPA filter. When operating, the chamber contained a relatively stable column of aerosol approximately 1 m in height and 20 cm in width from which the backscattering measurements were obtained. Filter samples collected from the aerosol chamber exhaust ports indicated that most of the aerosol particles were single cells approximately 1 micron in diameter. Given the measured chamber exhaust flow rate of 1000 liters/min, a 0.1% syringe pump fluid concentration resulted in an aerosol particle number density of approximately 10^7 particles/liter.

IR Backscatter Measurements

The backscattering measurements were performed using a grating tunable waveguide or low-pressure discharge CO_2 laser operating on approximately 50 lines covering the spectral range from 9.2 to 10.8 microns. A HeNe laser beam was coaligned with the CO_2 beam to facilitate alignment of the optical system. The resulting light beam

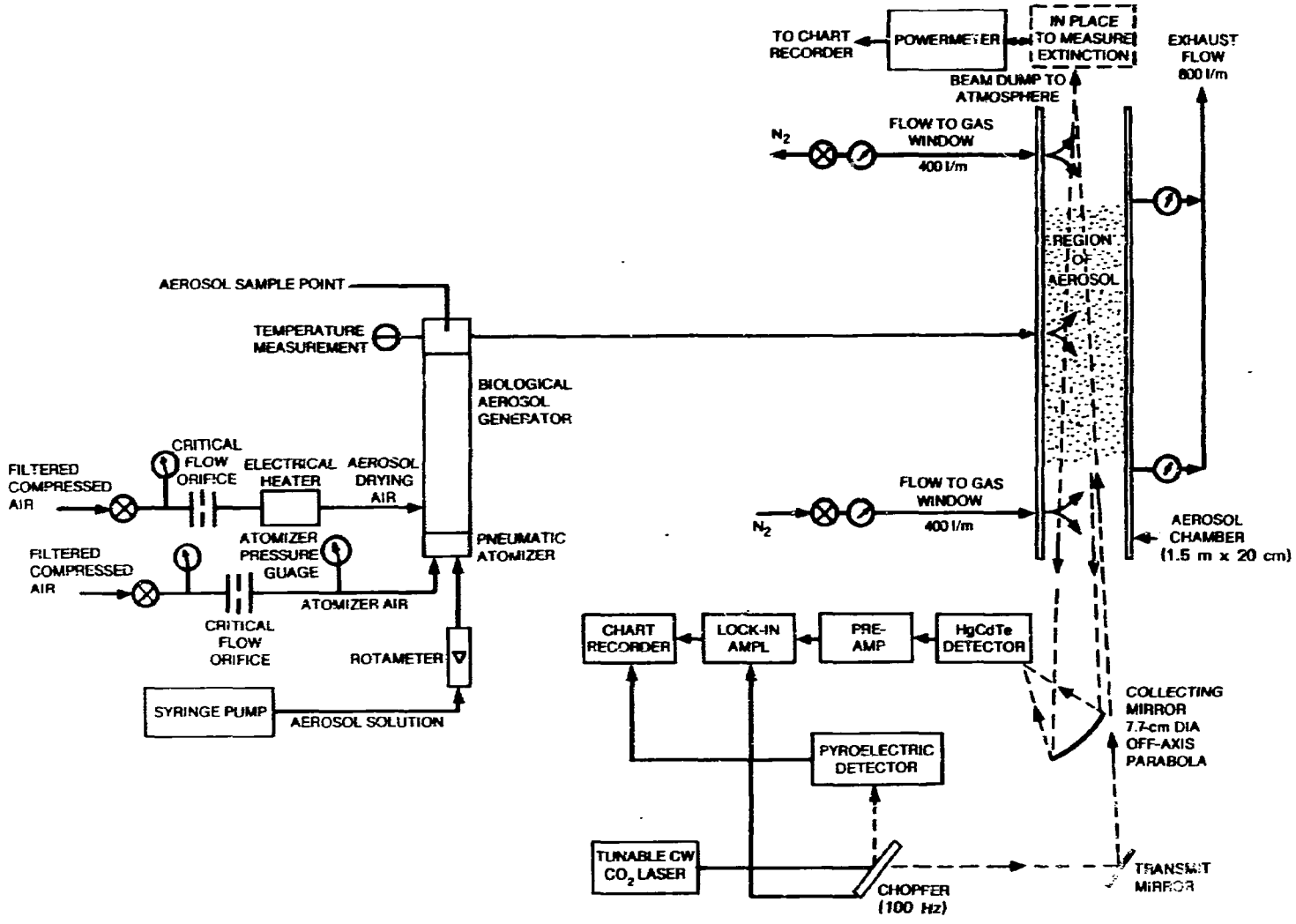

FIGURE 1 AEROSOL GENERATOR AND EQUIPMENT FOR MEASUREMENT OF 9- TO 11-μm BACKSCATTER AND EXTINCTION FROM BIOLOGICAL DISPERSIONS

was amplitude-modulated at 100 Hz by a copper-plated mechanical chopper, which deflected one-half of the beam power onto a thermopile detector. The detector output was monitored by a chart recorder, which allowed us to continuously monitor the transmitted laser power. The beam that passed through the chopper was directed via a transmit mirror into the aerosol column. The laser beam divergence angles were specified by the manufacturers to be 8.8 mrad for the waveguide laser and 3.5 mrad for the low-pressure discharge laser, and in either case the beam diameter at the aerosol chamber entrance was measured to be about 2 cm. Near backscattered light from the aerosol column was collected by a 7.7-cm diameter off-axis parabolic mirror and focused onto a 2 x 2-mm square HgCdTe detector manufactured by SBRC. The detector signal was amplified and fed into a lock-in amplifier referenced to the chopper frequency. The demodulated backscatter signal was monitored by a chart recorder along with the laser transmit power. A series of measurements at approximately 30 discrete CO_2 laser wavelengths were obtained on each aerosol sample with typical data collection times of 2 hours.

KBr Aerosols

To calibrate the optical system responsivity versus wavelength, our first backscattering measurements were performed on aerosols of KBr. This material was chosen because its complex refractive index in the 9- to 11-micron region is well known and essentially flat. It is also quite soluble in water and could therefore be fed into the chamber using the syringe pump method discussed previously. Finally, it was empirically found to form aerosols consisting predominantly of spherical particles, which allowed for a comparison of experimental data with Mie theory predictions.

The measurements were performed on KBr aerosols generated using a 1% concentration solution in the syringe pump. A particle cascade impactor was used to measure the aerosol size distribution at the center of the chamber. The size distribution was found to be log-normal with a

mass-median-diameter of 0.9 micron with a geometric standard deviation of 2.49.

These parameters were fed into a Mie code along with the KBr refractive indices and the expected backscattering signature was computed over the 9.2- to 10.8-micron region. The theoretical results were normalized at 10.6 microns and plotted as the solid curve in Figure 2. As a result of the behavior of the KBr refractive index, the backscatter curve is essentially flat and therefore forms a good system calibration baseline. The triangles in the figure denote our measured backscatter data from KBr aerosols. These data were collected using the waveguide laser and are simply the recorded backscatter signal at a given wavelength divided by the transmitted laser power at the same wavelength and normalized to the result at 10.6 microns (10P20 line).

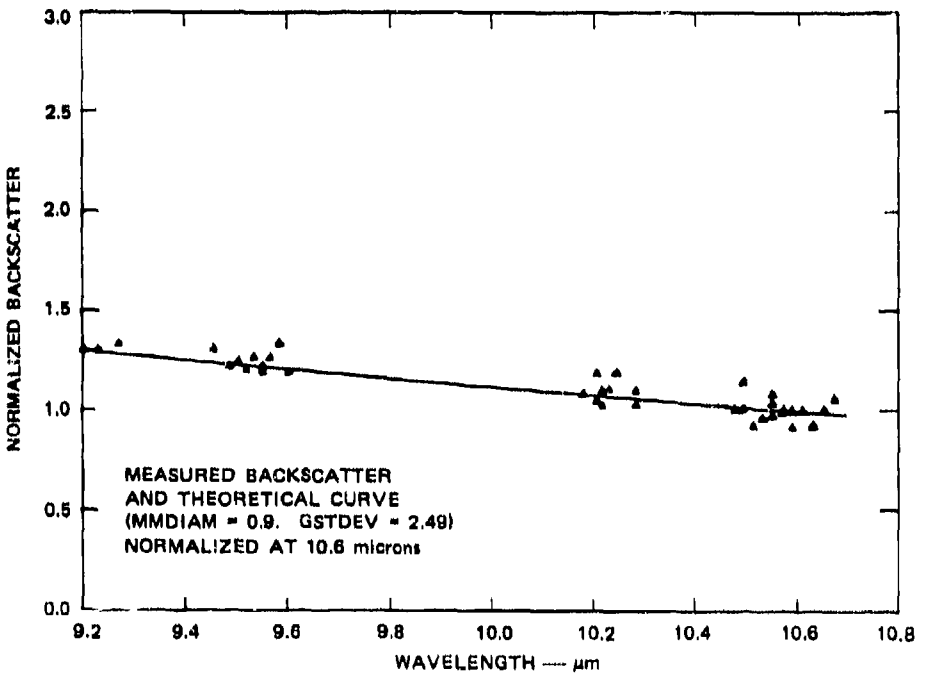

FIGURE 2 NORMALIZED IR BACKSCATTER FROM KBr AEROSOL

The experimental data and theoretical curve are in excellent agreement. This is perhaps somewhat surprising, since the data are not corrected for the wavelength-dependent responsivity of the HgCdTe detector. The responsitivity is not yet known for our detector, but for many HgCdTe detectors, the responsivity can drop by as much as 40% from the 11- to 9-micron region. We are planning to resolve this puzzle by measuring the responsivity of our detector. However, if we take the KBr results as a system calibration curve, we see that for whatever reasons our detection system response is essentially flat to within experimental error in the 9- to 11-micron region. Consequently, all of our biological aerosol backscattering data are presented assuming a flat system response.

B. subtilis Spore Aerosols

Our measurements on aerosols of B. subtilis spores were initially performed using a 0.1% concentration solution in the syringe pump. The aerosols generated were measured with the cascade impactor as log-normal with a 1.15-micron mass-median-diameter and a geometric standard deviation of 2.

Theoretical calculations were performed using our Mie code, these size distribution parameters, and our measured complex refractive index data for B. subtilis spores. The theoretical results are plotted as the solid curve in Figure 3 normalized at 10.6 microns. As before, the triangles denote the experimental data points obtained by calculating the ratio of the backscatter signal at any given wavelength to the transmitted laser power at that same wavelength and finally normalizing all data points to the result at 10.6 microns. In the 10-micron region, the data are in reasonable agreement with the Mie theory calculation, which is perhaps not surprising since both are normalized at 10.6 microns. There is a slight discrepancy in the 9-micron region, which may be due to the laser mode structure. In this region, the waveguide laser displayed a tendency to oscillate in the TEM_{01} mode ("donut" mode), rather than in the TEM_{00} mode, and we found empirically that at any given wavelength the normalized backscatter was larger for the TEM_{01}

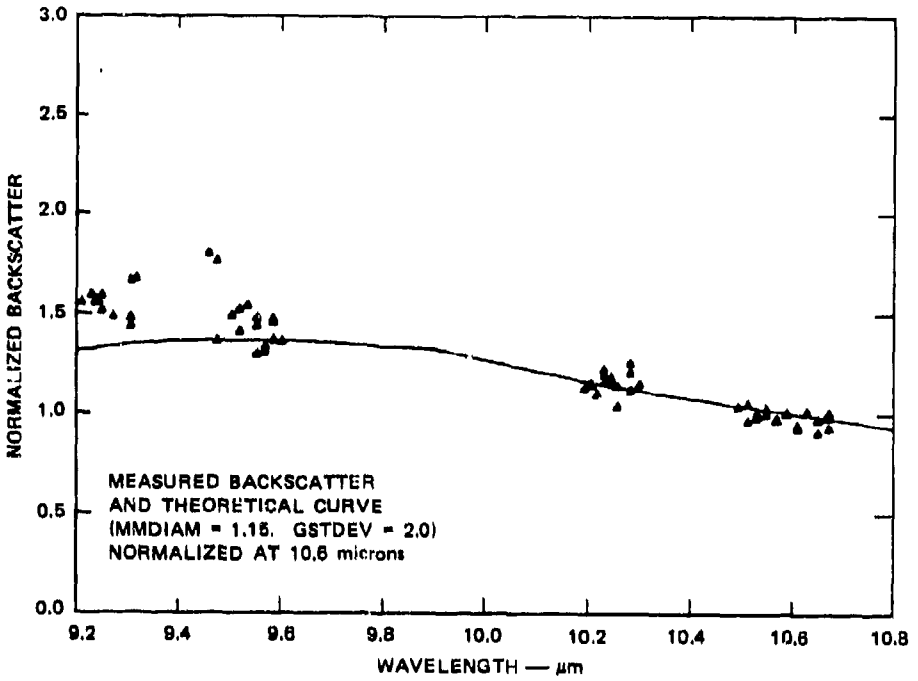

FIGURE 3 NORMALIZED IR BACKSCATTER FROM B. SUBTILIS SPORES AEROSOL (0.1% concentration)

than for the TEM_{00} mode. (For this reason, it was important to verify at each wavelength, using a thermal image plate, that the laser was indeed in the TEM_{00} mode.) The observed discrepancy probably resulted from the laser not exhibiting a clean mode structure in the 9-micron region.

Due to the problems with the waveguide CO_2 laser in the 9-micron region, we switched to a recently repaired low-pressure discharge CO_2 laser and repeated measurements on aerosols of B. subtilis spores using a 0.3% syringe pump concentration. This laser was considerably easier to tune and exhibited good TEM_{00} mode structure over the 9- and 10-micron bands. We chose to use a 0.3% solution concentration because more material was available for this run, and we found empirically that this increase in concentration affected only the aerosol particle number density and not the size distribution. Consequently, the aerosol backscatter signal was about a factor of 3 larger for the 0.3% case than for the 0.1% case. The results are plotted along with the theoretical curve in Figure 4, and we conclude that the theoretical Mie calculations based on our measured size distribution and complex refractive index for

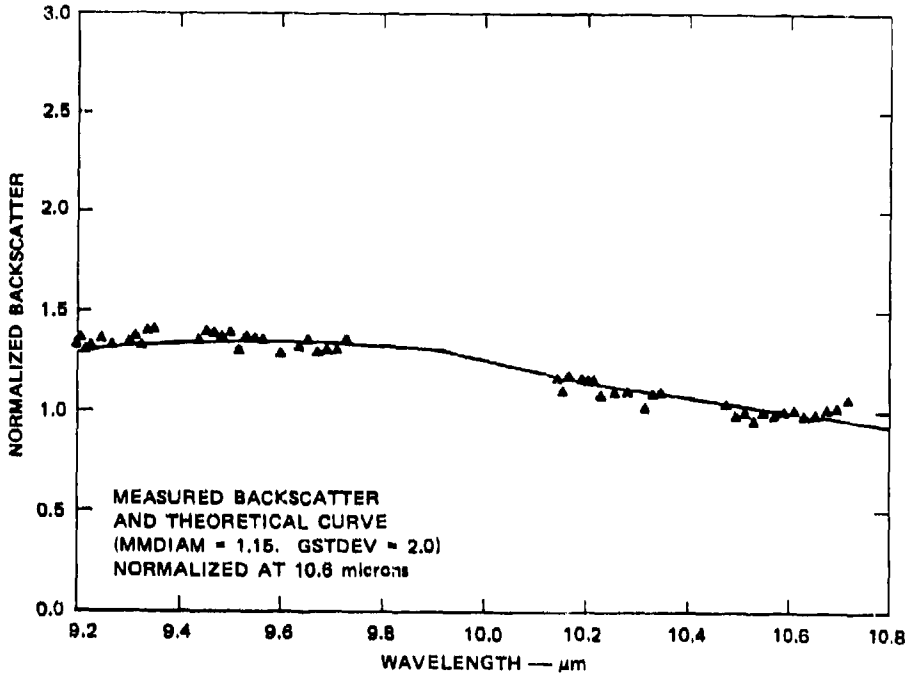

FIGURE 4 NORMALIZED IR BACKSCATTER FROM B. SUBTILIS SPORES AEROSOL (0.3% concentration)

B. subtilis spores are an excellent description of the experimental results.

B. subtilis Aerosols

We performed measurements on two different aerosol samples of the vegetative form of B. subtilis. Again, due to the limited amount of material available, these measurements were performed using 0.1% syringe pump solution concentration. Because of the perishable nature of the vegetative samples, all measurements were performed within 2 hours after receipt of the sample. Measurements on both samples were made with our waveguide laser and are summarized in Figure 5.

The theoretical curve on Figure 5 was generated as before by using our measured size distribution parameters and complex refractive index data in a Mie code. The large amount of scatter in the data is due to the fact that the backscatter signal from these samples was quite weak resulting in a rather marginal signal-to-noise ratio. Nevertheless, the

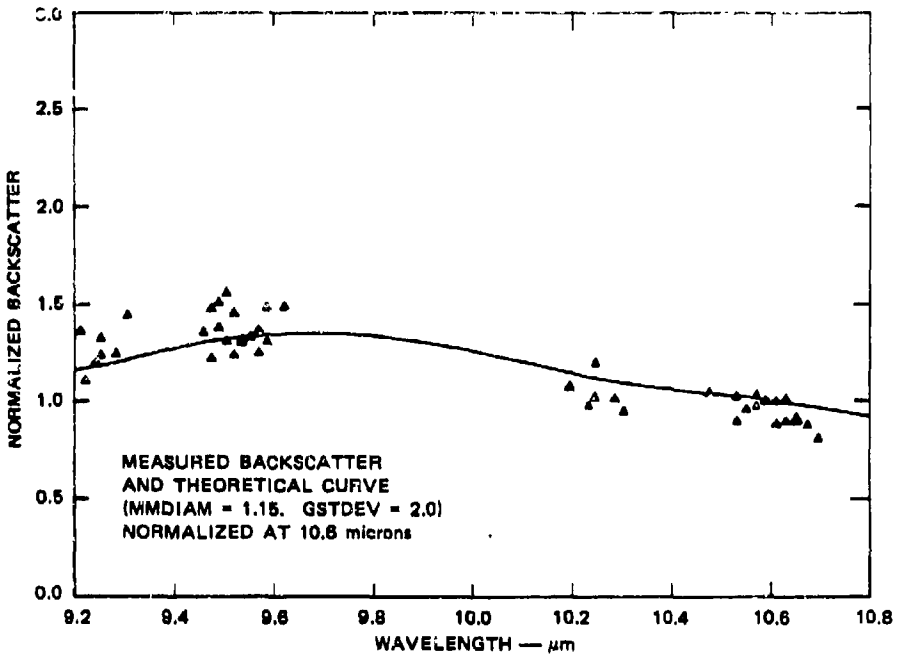

FIGURE 5 NORMALIZED IR BACKSCATTER FROM B. SUBTILIS AEROSOL

theoretical curve provides an adequate description of the measured backscatter. We did not attempt to repeat measurements using our low-pressure discharge CO_2 laser and a higher aerosol number density.

M. luteus Aerosols

Two sets of measurements were made on aerosols of M. luteus. The first series was performed using the waveguide laser and a 0.1% concentration syringe pump solution. The size distribution for these aerosols was measured to be log-normal with a 1.17-micron mass-median-diameter and a geometric standard deviation of 1.84.

In addition, filter samples were collected from one of the exit ports of the aerosol chamber. Figure 6 shows some scanning electron micrographs of the filter samples. We observe that the M. luteus cocci are approximately 0.7-micron diameter spheres and that most of our aerosol consists of clusters of at least two or more cocci.

Backscattering data collected from these aerosols are summarized in Figure 7 along with the theoretical Mie curve. The triangles represent

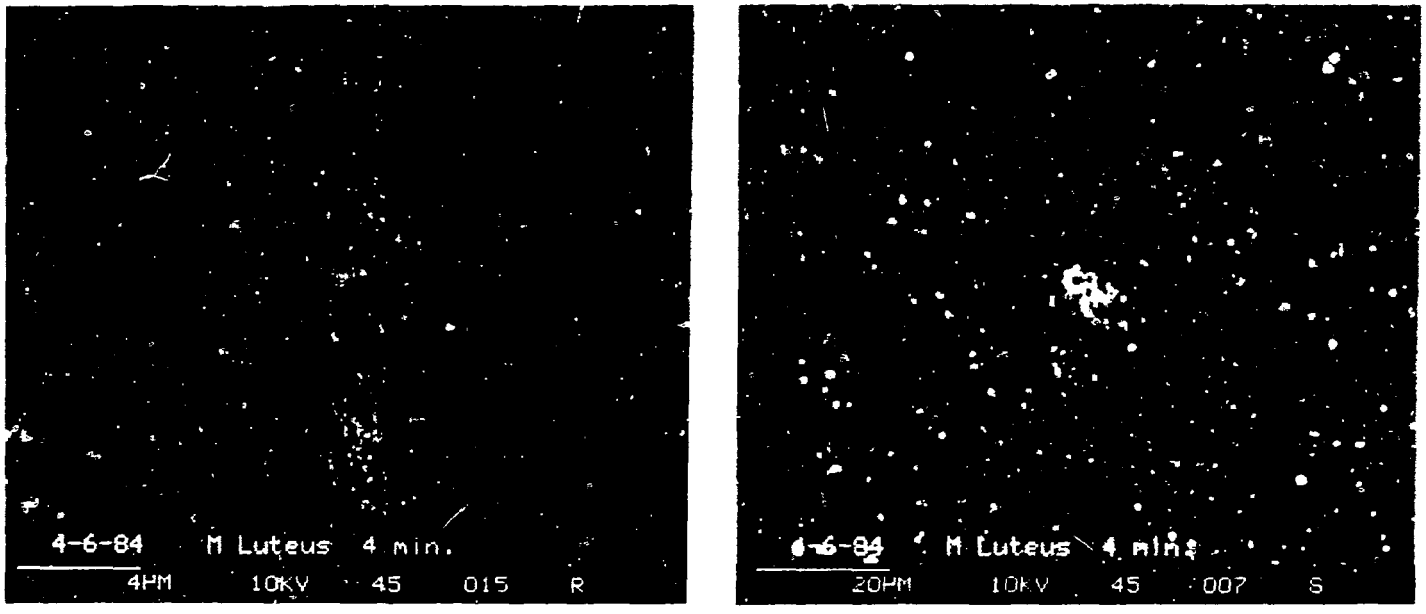

FIGURE 6 SCANNING ELECTRON MICROGRAPHS OF MICROCOCCUS LUTEUS
(samples taken from aerosol chamber)

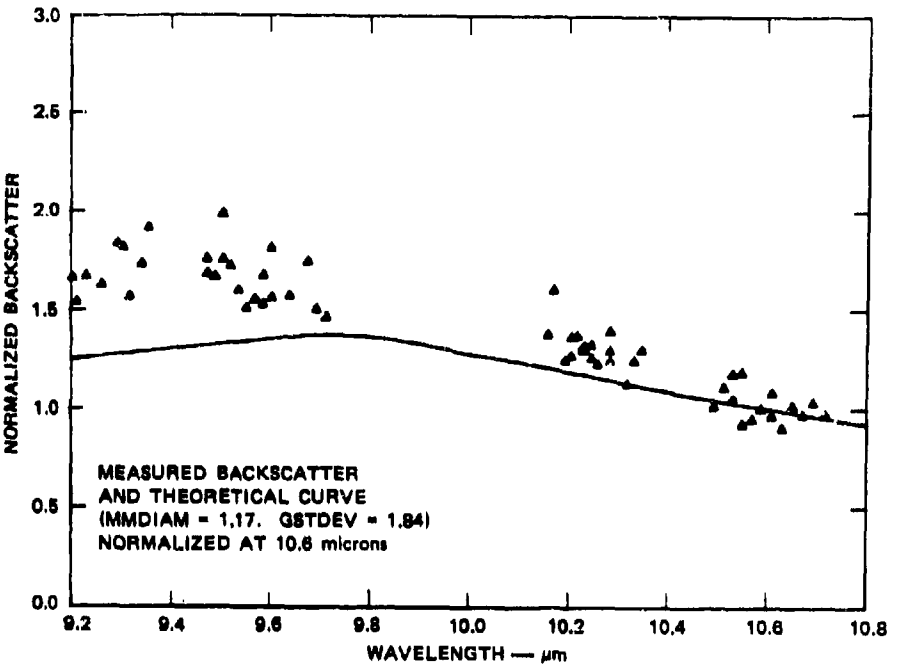

FIGURE 7 NORMALIZED IR BACKSCATTER FROM M. LUTEUS AEROSOL (0.1% concentration)

data collected on two separate M. luteus samples. The backscatter signal from these aerosols was quite small and resulted in the lowest signal-to-noise ratio of all our aerosol data. Therefore, the amount of scatter in the data is considerable. As was noted for the 0.1% B. subtilis spores data, we observed a systematic deviation of experiment from the theory that is especially pronounced in the 9-micron region. The origin of this deviation is not clear, but, as before, it may be due to the impure mode structure of the waveguide laser in this region.

Because of this deviation, we performed an additional set of M. luteus measurements using the low-pressure discharge CO_2 laser and a 0.3% concentration syringe pump solution to increase the aerosol particle number density and therefore the aerosol backscatter signal. These data are limited because we did not have enough material to make a complete series of measurements and we were also unable to measure the aerosol size distribution. However, based upon our results with B. subtilis spores, we do not expect the size distribution to be

significantly affected by the slight increase in solution concentration. Our data are plotted in Figure 8 along with a theoretical curve generated using the size distribution parameters measured on the 0.1% aerosol Clearly, there are no inconsistencies between theory and our measured backscatter.

Conclusions

We have measured IR backscatter in the 9- to 11-micron region from aerosols of B. subtilis spores, B. subtilis, and M. luteus using a tunable CO_2 laser. The data are consistent with the prediction of Mie theory using the measured complex refractive indices appropriate to the material and the measured aerosol size distribution parameters. Apparently, in the spectral region considered, the IR backscatter is quite insensitive to the shape of the particles, since the Mie predictions work equally well for B. subtilis (prolate ellipsoid, bacilli) and M. luteus (spherical, cocci). We also note that the 9- to 11-micron backscatter signatures for aerosols of these materials are rather uninteresting and exhibit no characteristic feature that might be used for DISC detection. Also, as illustrated in Figure 9 for B. subtilis spores, the backscatter curves are rather insensitive to the actual aerosol size distribution considered. Based on these results, we conclude that under optimum circumstances an atmospheric biological aerosol would be detectable using IR DISC only if its concentration exceeded 100 particles/cm^3. Considering that the U.S. Army's biological aerosol particle detection sensitivity goal is in the range of 5 to 10 particles/liter, we conclude that IR DISC is not a viable technique. SRI presented these results and conclusions at the Third Biodetection Workshop, April 25-26, 1984, sponsored by the U.S. Army Research Office.

3. Future Plans

Due to its inherently poor sensitivity for biological aerosol detection, we propose to delete the IR DISC work from the current contract. In its place, we propose to proceed with a more promising differential scattering detection concept that may meet the Army's stated detection sensitivity goals. The concept is known as circular-

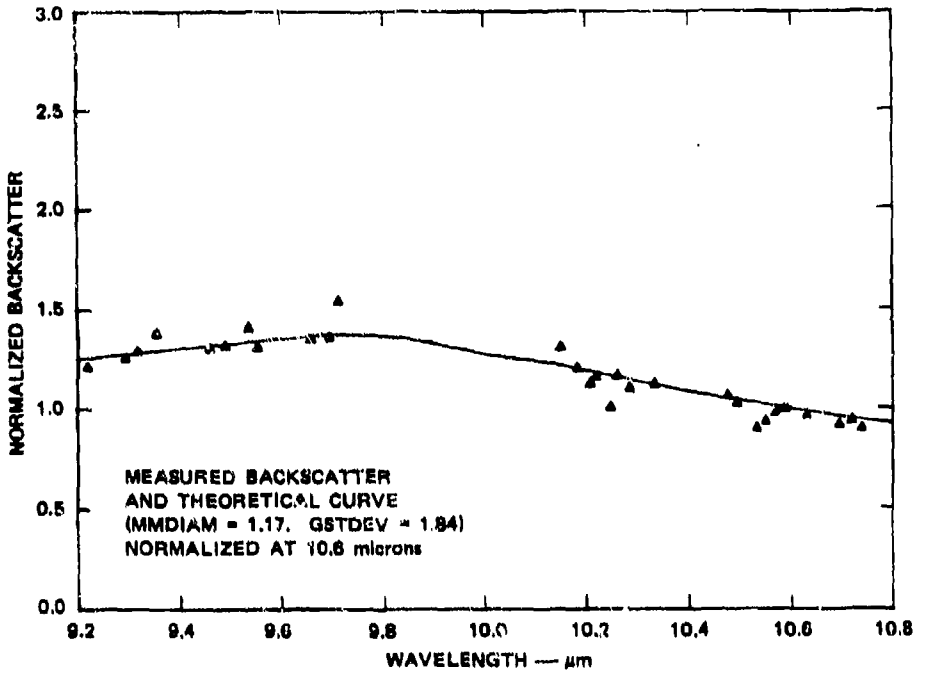

FIGURE 8 NORMALIZED IR BACKSCATTER FROM M. LUTEUS AEROSOL (0.3% concentration)

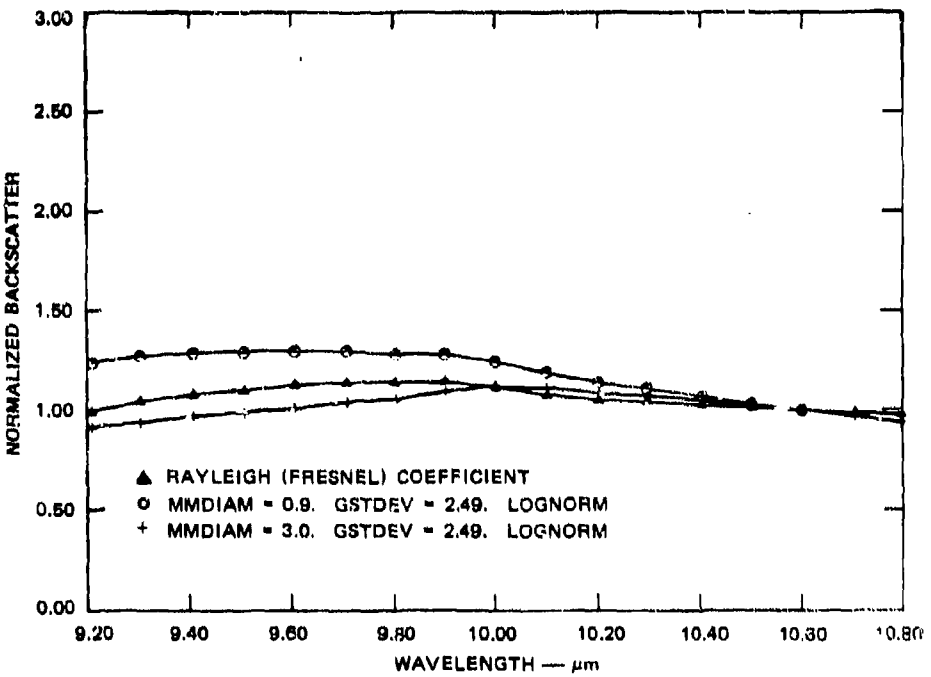

FIGURE 9 EFFECT OF SIZE DISTRIBUTION ON IR BACKSCATTERING FROM B. SUBTILIS SPORES AEROSOLS

intensity differential scattering (CIDS). The effect is essentially the differential scattering of left and right circular polarized light from particles of biological material and results from the chiral structure of biological macromolecules. CIDS signals from viruses and bacteria range from 10^{-3} to 10^{-4} in the 300- to 600-nm region, and the spectra provide unique signatures for the identification of microorganisms.

We propose to pursue CIDS from biological aerosols using the experimental configuration illustrated in Figure 10. Our existing aerosol generation system and chamber will be used to generate and contain the simulant aerosol (most likely E. coli or B. subtilis spores). The optical system will consist entirely of SRI-owned or purchased equipment. The light source will be either a krypton-ion laser with discrete emission lines covering the 0.5- to 0.8-micron region or a xenon lamp and monochrometer with continuous spectral coverage from 0.35 to 1.1 microns. Since it is of greatest interest to collect spectra over as broad a range as possible, the first measurements will probably be made with the xenon source. The light will be transmitted to the aerosol through a broadband polarizing beamsplitter (Glan-Thompson prism), a fused silica photoelastic polarization modulator, and finally through a 12-cm aperture transmit/receive telescope. The photoelastic modulator will be used to modulate the polarization state of the transmitted light between left- and right-circular at a 50-kHz rate. As a result, any circular differential backscatter from the aerosol column will be detected as a 50-kHz intensity modulation using a silicon photodiode and a lock-in amplifier referenced to the modulator. As shown in Figure 10, the backscattered light is collected by the 12-cm telescope, passed a second time through the modulator and, as a result of this second pass, deflected by the polarizing beamsplitter onto the photodiode. The normalized CIDS signal is obtained by dividing the 50-kHz photocurrent component by the dc component. An attractive feature of this detection system is that it is essentially identical to a proposed fieldable instrument, which could perform either active or passive CIDS measurements on atmospheric aerosols as we discussed at the Biodetection Workshop.

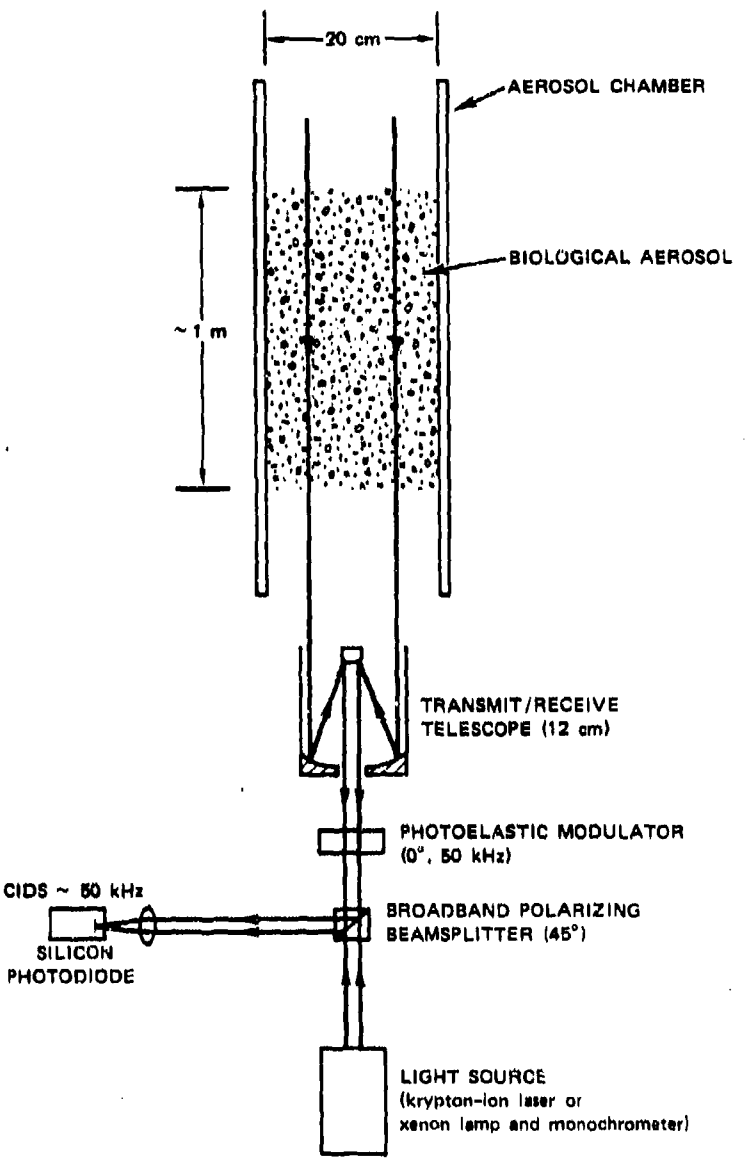

FIGURE 10 DIAGRAM OF EQUIPMENT FOR MEASUREMENT OF CIRCULAR INTENSITY DIFFERENTIAL SCATTERING FROM BIOLOGICAL SIMULANT AEROSOLS

A formal task proposal outlining the proposed change in program direction is now in preparation and will be sent to ARO for approval before we proceed with any CIDS work.

4. Scientific Personnel

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Diane D. Powell, M.S.
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